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[^{35}S]-Sulphate Uptake by *Xenopus laevis* Cartilage: The Influence of Plasma from the Growth Hormone-Treated Animal

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ABSTRACT—Plasma samples prepared from hypophysectomized *Xenopus laevis* which had received ovine or bullfrog GH injections stimulate the uptake of [^{35}S]-sulphate by the xiphisternal cartilage from the hypophysectomized *Xenopus* juveniles *in vitro*. The stimulating effect of the plasma is dependent on the dosage of GH which had been given to the plasma donors. Addition of GH to the incubation medium does not affect the uptake of labeled sulphate by the cartilage. The results indicate that GH induces the factor(s) which acts on the tissue to stimulate growth in the toad.

INTRODUCTION

It is widely accepted that in mammals, the growth-stimulating effect of GH is mediated by GH-dependent serum factor(s), somatomedins or insulin-like growth factors [1–3]. In amphibians, the growth-promoting activity of GH has been assessed by the increase in body weight, body length, femur length [4–7] or by the enhancement of the uptake of labeled sulphate by the cartilage [8]. Hypophysectomy results in the retardation of body growth and the decline of the uptake of labeled sulphate by the cartilage, which are restored by the GH supplementation [8, 9]. However, there is not much information of the mediator of somatotropic effect of GH in amphibians. The presence of somatomedin-like substance(s) in the serum of *Bufo marinus* has been evidenced immunologically by Daughaday *et al.* [10]. Rothstein *et al.* [11] demonstrated that administration of bovine GH to the hypophysectomized *Rana catesbeiana* increases the immunoreactive somatomedin C in the blood. In the present paper, we report that the plasma from the hypophysectomized *Xenopus laevis* treated with ovine or bullfrog GH stimulates chondroitin sulphate synthesis in the xiphisternal cartilage *in vitro*, the stimulating effect of the plasma being dependent on the concentration of the plasma and the dosage of the hormone which had been given to the plasma donor, and that GH added to the plasma from the hypophysectomized animal dose not affect the chondroitin sulphate synthesis in the cartilage.

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MATERIALS AND METHODS

Animals

Juvenile African clawed toads, *Xenopus laevis* of both sexes were used. They were hypophysectomized and kept for 7 days in tap water at 22°C prior to the experiment. Food (*Tubifex*) was provided in surplus throughout the experiment.

Hormones and plasma samples

Ovine GH(NIH-GH-S11) was supplied from the NIH. Bullfrog GH was prepared as a by-product during purification of bullfrog prolactin [12] according to the method described elsewhere [13]. Each hormone was dissolved in 1% BSA and given to the hypophysectomized toad (about 20 g in body weight) intraperitoneally at 10–11 a.m. every other day for 5 days (3 injections in total). Daily

dose of GH was 5–20 μg per animal. Twenty hours after the last injection, the blood was collected in a heparinized tube by heart puncture. Plasma was separated by centrifugation. Approximately 300 μl of plasma from each animal was pooled and stored at −70°C until use.

Incorporation of [^{35}S]-sulphate into cartilage

Xiphisternal cartilage from hypophysectomized toads weighing about 8 g was dissected out and cut into 0.8–1.0 mm sections. Each sample consisting of cartilage sections from 2 animals was transferred to a glass vial containing 2 ml sterile 67% Eagle's MEM (Nissui Seiyaku Co.) supplemented with 18mM $NaHCO_3$, 5 mM HEPES and 4 μCi of [^{35}S]-sulphate (carrier-free, Radiochemical Center, Amersham). To the medium, plasma from the hormone-treated or vehicle-injected animals, or plasma from vehicle-injected animals supplemented with GH was added as a test substance. Incubation was performed at 23°C in a Dubnoff-metabolic shaking incubator gassed with 95%O_2-5%CO_2. After incubation, the cartilage sections were immersed in boiling water for 10 min. Sections were then transferred into a small basket made of stainless steel and washed in saturated sodium sulphate, running water and distilled water. After drying in an oven at 55°C for 1 hr, the sections were weighed and solubilized in 250 μl of 98% formic acid at 110°C in glass vials with caps. After solubilization, 2.5 ml scintillation fluid was added to the vial. The radioactivity was measured in a liquid scintillation counter (Aloka, LSC-700). Analysis of the labeled cartilage revealed that about 60–80% of the label is incorporated into chondroitin sulphates [8].

RESULTS

The cartilage sections from the hypophysectomized *Xenopus laevis* were incubated for varying time at 23°C in the medium containing 10% plasma from the vehicle-injected animals (control) or ovine GH-treated animals. For the initial 5 hr of incubation, there was no difference in the uptake of the [^{35}S]-sulphate between the cartilage sections cultured in the medium containing the plasma from the GH-treated animals and those cultured in the plasma from the vehicle-injected animals. At 20 hr of incubation, the uptake of [^{35}S]-sulphate by the cartilage sections cultured in the medium containing the plasma from the hormone-treated toads was more prominent than that by the cartilage sections cultured in the medium containing the plasma from the vehicle-injected toads (Fig. 1). In the subsequent experiments, the cartilage was incubated for 20 hr.

Effect of various concentrations of plasma from the ovine GH-treated animals and from the vehicle-injected animals on the uptake of [^{35}S]-sulphate by the cartilage was studied. The stimulating effect of the plasma from the GH-treated toads increased according to the amount of the plasma added to the medium. On the other hand, the uptake of the [^{35}S]-sulphate by the cartilage was scarcely affected by the concentration of the plasma from the vehicle-injected animals. In Figure 2, the uptake of [^{35}S]-sulphate by the cartilage in response to 2.5-10% plasma from the ovine

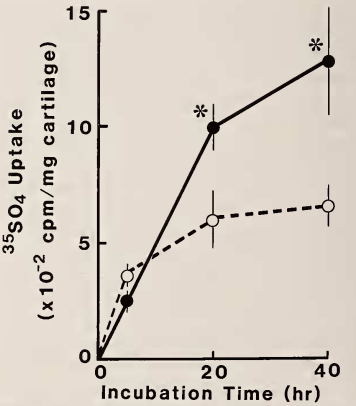

FIG. 1. Time course of the *in vitro* uptake of [^{35}S]-sulphate by the xiphisternal cartilage incubated in the medium containing 10% plasma from the ovine GH (10 μg/animal)-treated (●) or vehicle-injected (○) animals. Each point and vertical line represent the mean of 5 determinations and SEM, respectively.
*Statistically significant between the values for the 2 groups at $p<0.05$ (analysis of variance).

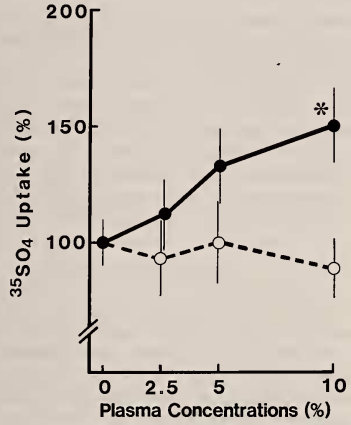

FIG. 2. Effect of various concentrations of the plasma from the ovine GH (10 μg/animal)-treated toads (●) and from the vehicle-injected animals (○) on the uptake of [^{35}S]-sulphate by the xiphisternal cartilage *in vitro*. The uptake of [^{35}S]-sulphate by the cartilage incubated for 20 hr with increasing concentrations of plasma is expressed as the percentage of the value for the cartilage incubated in the medium containing no plasma. Each point and vertical line represent the mean of 5 determinations and SEM, respectively.
*Statistically significant between the values for the 2 groups at $p<0.05$ (analysis of variance).

GH-treated and from the vehicle-injected animals is shown as the percentage of the value for the cartilage incubated in the medium containing no plasma. In the subsequent experiments, the medium containing 10% plasma was used unless otherwise stated.

The cartilage from the hypophysectomized toads was incubated for 20 hr in the medium containing 10% plasma taken from the animals which had been treated with various doses of ovine or bullfrog GH. The uptake of [^{35}S]-sulphate by the cartilage was enhanced by the plasma according to the dosage of GH given to the donor of the plasma (Fig. 3A and B). In order to confirm that chon-

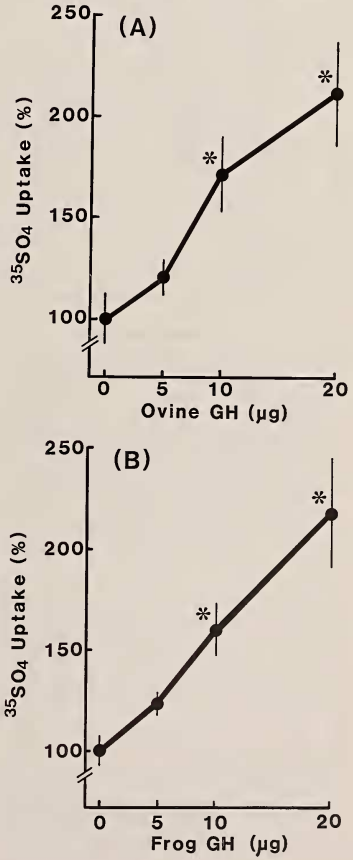

FIG. 3. Effect of plasma obtained from animals which had received various doses of ovine GH(A) or bullfrog GH(B) on the uptake of [^{35}S]-sulphate by the cartilage *in vitro*. The cartilage was incubated for 20 hr in the medium containing 10% plasma from the animals treated with 5–20 μg/animal of GH. The [^{35}S]-sulphate uptake is expressed as the percentage of the control value (the uptake in the medium containing the plasma from the animals treated with vehicle only). Each point and vertical line represent the mean of 5 determinations and SEM, respectively.
*Significantly different from the control (0 dose) value at $p<0.05$ (Student's *t*-test).

droitin sulphate synthesis in the cartilage of the donor of the plasma is also stimulated by GH, xiphisternal cartilage dissected out at the time of blood-taking was incubated in the medium containing [^{35}S]-sulphate for 12 hr. As shown in Figure 4, bullfrog GH administered *in vivo* caused a marked increase in the uptake of [^{35}S]-sulphate by the cartilage *in vitro*.

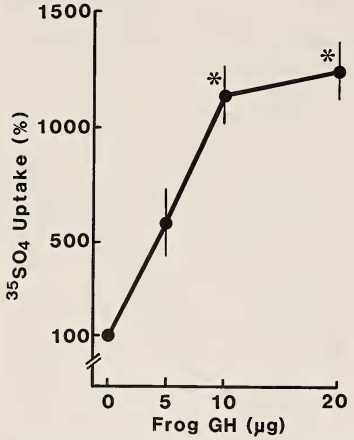

FIG. 4. *In vitro* uptake of [^{35}S]-sulphate by the xiphisternal cartilage from the animals which had received various doses of bullfrog GH. Incubation was performed for 12 hr according to the method of Ishii and Kikuyama [8]. [^{35}S]-sulphate uptake is expressed as the percentage of the control value. Each point and vertical line represent the mean of 5 determinations and SEM, respectively.
*Significantly different from the control (0 dose) value at $p<0.01$ (Cochran-cox test).

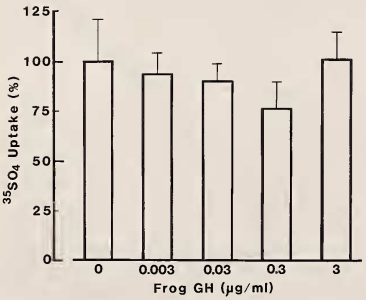

FIG. 5. Effect of bullfrog GH added in the medium on the uptake of [^{35}S]-sulphate by the xiphisternal cartilage *in vitro*. The cartilage was incubated for 20 hr in the medium containing 10% plasma from the vehicle-injected animals in the presence of various amount of bullfrog GH. Each column and vertical bar represent the mean of 4 determinations and SEM, respectively. No significant difference between the values for any 2 groups (Duncan's multiple range test).

In the final experiment, effect of GH added in the medium on the [^{35}S]-sulphate uptake by the xiphisternal cartilage was studied. [^{35}S]-sulphate uptake by the cartilage incubated in the medium containing 10% plasma from the vehicle-injected animals was not affected by addition of 0.003–3 μg of bullfrog GH per ml of the medium (Fig. 5).

DISCUSSION

Several investigators have reported that amphibian GHs stimulate growth in amphibians [6–9]. This is the first report communicating that amphibian GH induces the plasma factor(s) which stimulates the synthesis of chondroitin sulphates in the cartilage as mammalian GHs do. In the present experiment, bullfrog GH and ovine GH exhibited an equipotent activity in inducing plasma factor(s) in *Xenopus laevis*. It has been known that amino acid composition of amphibian GHs closely resemble that of mammalian GHs [13, 14]. According to our analytical data, amino acid sequences of 40 residues of NH_2-terminal and of COOH-terminal of bullfrog GH have 73 and 68% identity with the comparable portions of ovine GH, respectively [13].

Shapiro and Pimstone [15] observed that the plasma from the normal *Xenopus laevis* as well as several nonmammalian vertebrates enhances [^{35}S]-sulphate uptake by the porcine cartilage *in vitro*. However, they did not show that the stimulating factor(s) in the plasma is dependent on GH. We have previously observed that hypophysectomy of *Xenopus laevis* juveniles brings about a marked decline of incorporation of [^{35}S]-sulphate into the xiphisternal cartilage and that injection of GH

enhances the [^{35}S]-sulphate uptake by the cartilage dose-dependently [8]. The results of the present experiments indicate that the effect of GH on the cartilage is mediated by the plasma factor(s), because the stimulatory effect of the plasma was enhanced by GH which had been given to the plasma donors, while GH itself did not stimulate the *in vitro* [^{35}S]-sulphate uptake by the cartilage. In the present experiments, the maximum concentration of GH added to the incubation medium was 3 μg per ml. Plasma samples were obtained from the hypophysectomized animals which had received GH in total dose of 0.75–3 μg per g body weight. Therefore, the amount of GH added to the incubation medium containing the plasma from the hypophysectomized toads seems to be enough to substitute for the amount of GH existing in the incubation medium which contains 10% plasma from the GH-treated hypophysectomized animals. According to Van Buskirk *et al.* [16], hypophysectomy in *Rana catesbeiana* and *Rana pipiens* brings about the cessation of mitosis in the lens epithelium. Administration of GH reinitiates mitosis in the lens epithelium [17]. In organ cultures of lenses from the hypophysectomized frogs, the addition of serum from the hypophysectomized frogs treated with GH or normal frogs triggers cell division. However, the addition of serum from the hypophysectomized frogs does not stimulate cell proliferation. [18]. Rothstein *et al.* [11] demonstrated that immunoreactive somatomedin C existing in the serum of *Rana catesbeiana* was diminished by hypophysectomy and restored by GH administration and that administration of human somatomedin C to hypophysectomized bullfrogs induced mitosis in the lens epithelium. The results indicate that somatomedin C-like factor(s) mediates the GH-induced proliferation of the lens epithelium in bullfrogs. Although the presence of immunoreactive somatomedin C in amphibians is known only in two species such as *Rana catesbeiana* [11] and *Bufo marinus* [10], it is probable that the similar growth factor(s) mediates the action of GH in *Xenopus laevis* as observed in the present experiments.

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